PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF RECEIPT OF RECORD COPY

(PCT Rule 24.2(a))

To:

AOYAMA, Tamotsu
Aoyama & Partners, IMP Building
3-7, Shiromi 1-chome
Chuo-ku
Osaka-shi
Osaka 540-0001
JAPON

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
07 December 1998 (07.12.98)	
Applicant's or agent's file reference	**International application No.**
660857	PCT/JP98/05238

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

SAGAMI CHEMICAL RESEARCH CENTER et al (for all designated States except US)
KATO, Seishi (for US)

International filing date	:	20 November 1998 (20.11.98)
Priority date(s) claimed	:	25 November 1997 (25.11.97)
Date of receipt of the record copy by the International Bureau	:	04 December 1998 (04.12.98)
List of designated Offices	:	

EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
National :AU,CA,JP,MX,US

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

- [X] time limits for entry into the national phase
- [X] confirmation of precautionary designations
- [X] requirements regarding priority documents

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:

M. Sakai

Telephone No. (41-22) 338.83.38

002411005

INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated in the Notification of Receipt of Record Copy (Form PCT/IB/301) by paying national fees and furnishing translations, as prescribed by the applicable national laws.

The time limit for performing these procedural acts is **20 MONTHS** from the priority date or, for those designated States which the applicant elects in a demand for international preliminary examination or in a later election, **30 MONTHS** from the priority date, provided that the election is made before the expiration of 19 months from the priority date. Some designated (or elected) Offices have fixed time limits which expire even later than 20 or 30 months from the priority date. In other Offices an extension of time or grace period, in some cases upon payment of an additional fee, is available.

In addition to these procedural acts, the applicant may also have to comply with other special requirements applicable in certain Offices. **It is the applicant's responsibility** to ensure that the necessary steps to enter the national phase are taken in a timely fashion. Most designated Offices do not issue reminders to applicants in connection with the entry into the national phase.

For detailed information about the procedural acts to be performed to enter the national phase before each designated Office, the applicable time limits and possible extensions of time or grace periods, and any other requirements, see the relevant Chapters of Volume II of the PCT Applicant's Guide. Information about the requirements for filing a demand for international preliminary examination is set out in Chapter IX of Volume I of the PCT Applicant's Guide.

GR and ES became bound by PCT Chapter II on 7 September 1996 and 6 September 1997, respectively, and may, therefore, be elected in a demand or a later election filed on or after 7 September 1996 and 6 September 1997, respectively, regardless of the filing date of the international application. (See second paragraph above.)

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

CONFIRMATION OF PRECAUTIONARY DESIGNATIONS

This notification lists only specific designations made under Rule 4.9(a) in the request. It is important to check that these designations are correct. Errors in designations can be corrected where precautionary designations have been made under Rule 4.9(b). The applicant is hereby reminded that any precautionary designations may be confirmed according to Rule 4.9(c) before the expiration of 15 months from the priority date. If it is not confirmed, it will automatically be regarded as withdrawn by the applicant. There will be no reminder and no invitation. Confirmation of a designation consists of the filing of a notice specifying the designated State concerned (with an indication of the kind of protection or treatment desired) and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 16 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 16-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 16-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity to furnish the priority document within a time limit which is reasonable under the circumstances.

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 16-month time limit is the filing date of the earliest application whose priority is claimed.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

受付 11.1.25 (山口特許事務所)

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

AOYAMA, Tamotsu
Aoyama & Partners, IMP Building
3-7, Shiromi 1-chome
Chuo-ku
Osaka-shi
Osaka 540-0001
JAPON

Date of mailing (day/month/year) 19 January 1999 (19.01.99)	
Applicant's or agent's file reference 660857	**IMPORTANT NOTIFICATION**
International application No. PCT/JP98/05238	International filing date (day/month/year) 20 November 1998 (20.11.98)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 25 November 1997 (25.11.97)
Applicant SAGAMI CHEMICAL RESEARCH CENTER et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
25 Nove 1997 (25.11.97)	9/323129	JP	15 Janu 1999 (15.01.99)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

K. Takeda

Telephone No. (41-22) 338.83.38

002434397

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

AOYAMA, Tamotsu
Aoyama & Partners, IMP Building
3-7, Shiromi 1-chome
Chuo-ku
Osaka-shi
Osaka 540-0001
JAPON

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

受付 11.4 15

Date of mailing (day/month/year) 08 April 1999 (08.04.99)	
Applicant's or agent's file reference 660857	**IMPORTANT NOTIFICATION**
International application No. PCT/JP98/05238	**International filing date** (day/month/year) 20 November 1998 (20.11.98)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address

YAMAGUCHI, Tomoko
5-13-11, Takasago
Katsushika-ku
Tokyo 125-0054
Japan

State of Nationality	State of Residence
JP	JP

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address

KIMURA, Tomoko
302, 4-1-28, Nishiikuta
Tama-ku
Kawasaki-shi
Kanagawa 214-0037
Japan

State of Nationality	State of Residence
JP	JP

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[X] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer M. Sakai Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

002560665
外国方式

PATENT COOPERATION TREATY

受付 11.7.26

From the INTERNATIONAL BUREAU

PCT

INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

To:

AOYAMA, Tamotsu
Aoyama & Partners, IMP Building
3-7, Shiromi 1-chome
Chuo-ku
Osaka-shi
Osaka 540-0001
JAPON

Date of mailing (day/month/year)	
09 July 1999 (09.07.99)	
Applicant's or agent's file reference	**IMPORTANT INFORMATION**
660857	

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/JP98/05238	20 November 1998 (20.11.98)	25 November 1997 (25.11.97)

Applicant
SAGAMI CHEMICAL RESEARCH CENTER et al

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

   EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
   National :AU,CA,JP,US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

   National :MX

3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing , if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

   Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

   The entry into the European regional phase is postponed **until 31 months from the priority date** for all States designated for the purposes of obtaining a European patent.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:

Sean Taylor

Telephone No. (41-22) 338.83.38

2722307

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
660857		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/JP 98/05238	20/11/1998	25/11/1997
Applicant		
SAGAMI CHEMICAL RESEARCH CENTER et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readble form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP 98/05238

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see sheet B

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-6 in part (subject 1. on continuation-sheet)

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-6 in part

Protein comprising SEQ ID NO:1, DNA encoding it, cDNA comprising SEQ ID NO:4 or 7, expression vector and transformed eukaryotic cell capable of expressing the same.

2. Claims: 1-6 in part

Protein comprising SEQ ID NO:2, DNA encoding it, cDNA comprising SEQ ID NO:5 or 9, expression vector and transformed eukaryotic cell capable of expressing the same.

3. Claims: 1-6 in part

Protein comprising SEQ ID NO:3, DNA encoding it, cDNA comprising SEQ ID NO:6 or 11, expression vector and transformed eukaryotic cell capable of expressing the same.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP 98/05238

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 C12N15/85 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JULIE E. REEVES ET AL.: "The surf-4 gene encodes a novel 30kDa intgral membrane protein" MOLECULAR MEMBRANE BIOLOGY, vol. 12, no. 2, April 1995 (1995-04), pages 201-208, XP002096695 page 207, left-hand column, paragraph 1 - right-hand column, last paragraph; figure 1 --- -/--	1-6

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 March 1999	14. 07. 99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	MONTERO LOPEZ B.

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLARE HUXLEY ET AL.: "The mouse surfeit locus contains a cluster of six genes associated with four CpG-rich islands in 32 kilobases of genomic DNA" MOLECULAR AND CELLULAR BIOLOGY, vol. 10, no. 2, February 1990 (1990-02), pages 605-614, XP002096696 WASHINGTON US figure 2 ---	1-6
A	YU FENG ET AL.: "HIV-1 entry cofactor: Functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor" SCIENCE, vol. 272, no. 5263, 10 May 1996 (1996-05-10), pages 872-877, XP002096721 cited in the application the whole document -----	1-6

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D6

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 09 July 1999 (09.07.99)	
International application No. PCT/JP98/05238	**Applicant's or agent's file reference** 660857
International filing date (day/month/year) 20 November 1998 (20.11.98)	**Priority date** (day/month/year) 25 November 1997 (25.11.97)
Applicant KATO, Seishi et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:
09 June 1999 (09.06.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Sean Taylor

Telephone No.: (41-22) 338.83.38

2722308

Form PCT/IB/331 (July 1992)

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:
AOYAMA, Tamotsu
Aoyama & Partners, IMP Building
3-7, Shiromi 1-chome
Chuo-ku
Osaka-shi
Osaka 540-0001
JAPON

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

Date of mailing (day/month/year)	
03 June 1999 (03.06.99)	
Applicant's or agent's file reference 660857	**IMPORTANT NOTICE**

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/JP98/05238	20 November 1998 (20.11.98)	25 November 1997 (25.11.97)

Applicant
SAGAMI CHEMICAL RESEARCH CENTER et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,EP,JP,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   CA,MX

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   03 June 1999 (03.06.99) under No. WO 99/27094

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Form PCT/IB/308 (July 1996)

5-

PATENT COOPERATION TREATY

PCT

REC'D 09 FEB 2000
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
660857		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP98/05238	20/11/1998	25/11/1997
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant SAGAMI CHEMICAL RESEARCH CENTER et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
VI	☐	Certain documents cited
VII	☒	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09/06/1999	09.02.00
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Montero Lopez, B Telephone No. +31 70 340 3739

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/JP98/05238

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-57 as originally filed

Claims, No.:

1-6 as originally filed

Drawings, sheets:

1/3-3/3 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-6 as far as concerning a protein comprising SEQ ID NO:1, DNA encoding it, cDNA comprising SEQ ID NO:4 or 7, expression vector and transformed eukaryotic cell capable of expressing the same..

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 3, 4
	No:	Claims 1, 2, 5, 6
Inventive step (IS)	Yes:	Claims
	No:	Claims 1-6
Industrial applicability (IA)	Yes:	Claims 1-6
	No:	Claims

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item I

Basis of the opinion

Pages 1/26-26/26 of the sequence listing have been considered part of the description.

Re Item IV

Lack of unity of invention

The separate inventions are:

1. Protein comprising SEQ ID NO:1, DNA encoding it, cDNA comprising SEQ ID NO:4 or 7, expression vector and transformed eukaryotic cell capable of expressing the same.

2. Protein comprising SEQ ID NO:2, DNA encoding it, cDNA comprising SEQ ID NO:5 or 9, expression vector and transformed eukaryotic cell capable of expressing the same.

3. Protein comprising SEQ ID NO:3, DNA encoding it, cDNA comprising SEQ ID NO:6 or 11, expression vector and transformed eukaryotic cell capable of expressing the same.

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

The present application relates to human proteins having transmembrane domains. Multiple human proteins having transmembrane domains have been identified in the prior art as it is recognized in the application, description pages 1-2. Transmembrane proteins are described for example in "Science" 1996, vol. 272, no. 5263, pages 872-877, disclosing a HIV-1 entry cofactor which comprises seven-transmembrane domains, and in "Mol. Membrane Biol.", 1995, vol. 12, pages 201-208, disclosing an integral membrane protein. In the light of the prior art the problem underlying the present application can be formulated as providing further human proteins having

transmembrane domains. The following solutions are proposed:

1. Providing a protein comprising sequence SEQ ID NO:1 and DNA encoding it.

2. Providing a protein comprising sequence SEQ ID NO:2 and DNA encoding it.

3. Providing a protein comprising sequence SEQ ID NO:3 and DNA encoding it.

Given the essential difference between the primary structure of the polypeptides of sequences SEQ ID NO:1, 2 and 3 and their corresponding encoding nucleic acid sequences, due to the fact that human proteins with transmembrane domains have already been disclosed in the state of the art, and since in the light of the state of the art, no other technical feature could be distinguished as being new and common to the identified problem and proposed solutions, the requisite unity of invention (Rule 13.1 PCT) therefore no longer exists inasmuch as a technical relationship involving one or more of the same or corresponding special technical features in the sense of Rule 13.2 PCT does not exist between the subject-matter of the above mentioned groups of dependent claims.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: JULIE E. REEVES ET AL.: "The surf-4 gene encodes a novel 30kDa integral membrane protein" MOLECULAR MEMBRANE BIOLOGY, vol. 12, no. 2, April 1995 (1995-04), pages 201-208, XP002096695.

D2: CLARE HUXLEY ET AL.: "The mouse surfeit locus contains a cluster of six genes associated with four CpG-rich islands in 32 kilobases of genomic DNA" MOLECULAR AND CELLULAR BIOLOGY, vol. 10, no. 2, February 1990 (1990-02), pages 605-614, XP002096696 WASHINGTON US.

1. Documents D1 and D2 disclose the surf-4 gene, which encodes a 30kDa integral membrane protein sharing 100% sequence identity with Sequence No.1, as well as a vector comprising it and its production by Hela cells (see Experimental procedures in D1). Consequently, claims 1, 2, 5, and 6 are not novel and do not meet the requirements of Article 33(2) PCT.

2. Claims 3 and 4 relate to a cDNA comprising Sequences No.4 and 7. Since such cDNA has not been disclosed in the state of the art, claims 3 and 4 are novel and comply with the requirements of Article 33(2) PCT.

2.1. Document D1, which is considered to represent the most relevant state of the art, discloses the isolation of the coding region for the surf-4 gene. Document D1 differs only from the subject-matter of claims 3 and 4 in that the particular sequences Nos. 4 and 7 are not disclosed. However, the sequencing of an isolated gene is a routine procedure in the art, which the skilled person would perform without the need of exercising any inventive step. Consequently, claims 3 and 4 do not involve an inventive step and do not meet the requirements of Article 33(3) PCT.

Re Item VII

Certain defects in the international application

1. Claims 1, 3, and 4 contain references to the drawings. According to Rule 6.2(a) PCT, claims should not contain such references except where absolutely necessary, which is not the case here.

Re Item VIII

Certain observations on the international application

1. General statements in the description which imply that the extent of protection may be expanded in a not precisely defined way are not allowable. The inclusion of the

expression "hereby incorporated by reference" in the description contradicts therefore the requirements of Article 6.

2. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 and D2 is not mentioned in the description, nor are these documents identified therein.

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

受付 11.8.25 武山特許事務所

PCT

WRITTEN OPINION

(PCT Rule 66)

To:

Aoyama & Partners
IMP Building, 3-7, Shiromi
1-chome, Chuo-ku, Osaka-shi
Osaka 540-0001
JAPON

Date of mailing *(day/month/year)*	20. 08. 1999
Applicant's or agent's file reference: 660857	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP98/05238	20/11/1998	25/11/1997

International Patent Classification (IPC) or both national classification and IPC

C12N15/12

Applicant

SAGAMI CHEMICAL RESEARCH CENTER et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain document cited
VII	☒	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 25/03/2000.

Name and mailing address of the international preliminary examining authority:

European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. (+31-70) 340-2040 Tx: 31 651 epo nl
Fax: (+31-70) 340-3016

Authorized officer / Examiner
Montero Lopez, B

Formalities officer (incl. extension of time limits)
Kruydenberg, G
Telephone No. (+31-70)-340 2277

I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-57 as originally filed

Claims, No.:

1-6 as originally filed

Drawings, sheets:

1/3-3/3 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with for the following reasons

and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:

see separate sheet

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

☐ all parts.

☒ the parts relating to claims Nos. 1-6 as far as concerning a protein comprising SEQ ID NO:1, DNA encoding it, cDNA comprising SEQ ID NO:4 or 7, expression vector and transformed eukaryotic cell capable of expressing the same..

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1, 2, 5, 6
Inventive step (IS)	Claims	3, 4
Industrial applicability (IA)	Claims	

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item I
Basis of the opinion

Pages 1/26-26/26 of the sequence listing have been considered part of the description.

Re Item IV
Lack of unity of invention

The separate inventions are:

1. Protein comprising SEQ ID NO:1, DNA encoding it, cDNA comprising SEQ ID NO:4 or 7, expression vector and transformed eukaryotic cell capable of expressing the same.

2. Protein comprising SEQ ID NO:2, DNA encoding it, cDNA comprising SEQ ID NO:5 or 9, expression vector and transformed eukaryotic cell capable of expressing the same.

3. Protein comprising SEQ ID NO:3, DNA encoding it, cDNA comprising SEQ ID NO:6 or 11, expression vector and transformed eukaryotic cell capable of expressing the same.

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

The present application relates to human proteins having transmembrane domains. Multiple human proteins having transmembrane domains have been identified in the prior art as it is recognized in the application, description pages 1-2. Transmembrane proteins are described for example in "Science" 1996, vol. 272, no. 5263, pages 872-877, disclosing a HIV-1 entry cofactor which comprises seven-transmembrane domains, and in "Mol. Membrane Biol.", 1995, vol. 12, pages 201-208, disclosing an integral membrane protein. In the light of the prior art the problem underlying the present application can be formulated as providing further human proteins having

transmembrane domains. The following solutions are proposed:

1. Providing a protein comprising sequence SEQ ID NO:1 and DNA encoding it.

2. Providing a protein comprising sequence SEQ ID NO:2 and DNA encoding it.

3. Providing a protein comprising sequence SEQ ID NO:3 and DNA encoding it.

Given the essential difference between the primary structure of the polypeptides of sequences SEQ ID NO:1, 2 and 3 and their corresponding encoding nucleic acid sequences, due to the fact that human proteins with transmembrane domains have already been disclosed in the state of the art, and since in the light of the state of the art, no other technical feature could be distinguished as being new and common to the identified problem and proposed solutions, the requisite unity of invention (Rule 13.1 PCT) therefore no longer exists inasmuch as a technical relationship involving one or more of the same or corresponding special technical features in the sense of Rule 13.2 PCT does not exist between the subject-matter of the above mentioned groups of dependent claims.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: JULIE E. REEVES ET AL.: "The surf-4 gene encodes a novel 30kDa integral membrane protein" MOLECULAR MEMBRANE BIOLOGY, vol. 12, no. 2, April 1995 (1995-04), pages 201-208, XP002096695.

D2: CLARE HUXLEY ET AL.: "The mouse surfeit locus contains a cluster of six genes associated with four CpG-rich islands in 32 kilobases of genomic DNA" MOLECULAR AND CELLULAR BIOLOGY, vol. 10, no. 2, February 1990 (1990-02), pages 605-614, XP002096696 WASHINGTON US.

1. Documents D1 and D2 disclose the surf-4 gene, which encodes a 30kDa integral membrane protein sharing 100% sequence identity with Sequence No.1, as well as a vector comprising it and its production by Hela cells (see Experimental procedures in D1). Consequently, claims 1, 2, 5, and 6 are not novel and do not meet the requirements of Article 33(2) PCT.

2. Document D1, which is considered to represent the most relevant state of the art, discloses the isolation of the coding region for the surf-4 gene. Document D1 differs only from the subject-matter of claims 3 and 4 in that the particular sequences Nos. 4 and 7 are not disclosed. However, the sequencing of an isolated gene is a routine procedure in the art, which the skilled person would perform without the need of exercising any inventive step. Consequently, claims 3 and 4 do not involve an inventive step and do not meet the requirements of Article 33(3) PCT.

Re Item VII
Certain defects in the international application

1. Claims 1, 3, and 4 contain references to the drawings. According to Rule 6.2(a) PCT, claims should not contain such references except where absolutely necessary, which is not the case here.

Re Item VIII
Certain observations on the international application

1. General statements in the description which imply that the extent of protection may be expanded in a not precisely defined way are not allowable. The applicant is therefore kindly requested to delete the expression "hereby incorporated by reference" wherever it appears in the description (Article 6 PCT).

2. Pages 1/26-26/26 of the sequence listing should be renumbered as pages 57-83 of the description.

3. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 and D2 is not mentioned in the description, nor are these documents identified therein.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Aoyama & Partners
IMP Building, 3-7, Shiromi
1-chome, Chuo-ku, Osaka-shi
Osaka 540-0001
JAPON

受付
12. 2. 14
青山特許事務所

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	09.02.00
Applicant's or agent's file reference 660857	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP98/05238	20/11/1998	25/11/1997

Applicant
SAGAMI CHEMICAL RESEARCH CENTER et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Sinanovic, E Tel.+31 70 340-3596

Form PCT/IPEA/416 (July 1992)

外国方式

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
660857		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP98/05238	20/11/1998	25/11/1997
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant SAGAMI CHEMICAL RESEARCH CENTER et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09/06/1999	09.02.00
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Montero Lopez, B Telephone No. +31 70 340 3739

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/JP98/05238

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-57 as originally filed

Claims, No.:

1-6 as originally filed

Drawings, sheets:

1/3-3/3 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-6 as far as concerning a protein comprising SEQ ID NO:1, DNA encoding it, cDNA comprising SEQ ID NO:4 or 7, expression vector and transformed eukaryotic cell capable of expressing the same..

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 3, 4
	No:	Claims 1, 2, 5, 6
Inventive step (IS)	Yes:	Claims
	No:	Claims 1-6
Industrial applicability (IA)	Yes:	Claims 1-6
	No:	Claims

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item I

Basis of the opinion

Pages 1/26-26/26 of the sequence listing have been considered part of the description.

Re Item IV

Lack of unity of invention

The separate inventions are:

1. Protein comprising SEQ ID NO:1, DNA encoding it, cDNA comprising SEQ ID NO:4 or 7, expression vector and transformed eukaryotic cell capable of expressing the same.

2. Protein comprising SEQ ID NO:2, DNA encoding it, cDNA comprising SEQ ID NO:5 or 9, expression vector and transformed eukaryotic cell capable of expressing the same.

3. Protein comprising SEQ ID NO:3, DNA encoding it, cDNA comprising SEQ ID NO:6 or 11, expression vector and transformed eukaryotic cell capable of expressing the same.

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

The present application relates to human proteins having transmembrane domains. Multiple human proteins having transmembrane domains have been identified in the prior art as it is recognized in the application, description pages 1-2. Transmembrane proteins are described for example in "Science" 1996, vol. 272, no. 5263, pages 872-877, disclosing a HIV-1 entry cofactor which comprises seven-transmembrane domains, and in "Mol. Membrane Biol.", 1995, vol. 12, pages 201-208, disclosing an integral membrane protein. In the light of the prior art the problem underlying the present application can be formulated as providing further human proteins having

transmembrane domains. The following solutions are proposed:

1. Providing a protein comprising sequence SEQ ID NO:1 and DNA encoding it.

2. Providing a protein comprising sequence SEQ ID NO:2 and DNA encoding it.

3. Providing a protein comprising sequence SEQ ID NO:3 and DNA encoding it.

Given the essential difference between the primary structure of the polypeptides of sequences SEQ ID NO:1, 2 and 3 and their corresponding encoding nucleic acid sequences, due to the fact that human proteins with transmembrane domains have already been disclosed in the state of the art, and since in the light of the state of the art, no other technical feature could be distinguished as being new and common to the identified problem and proposed solutions, the requisite unity of invention (Rule 13.1 PCT) therefore no longer exists inasmuch as a technical relationship involving one or more of the same or corresponding special technical features in the sense of Rule 13.2 PCT does not exist between the subject-matter of the above mentioned groups of dependent claims.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: JULIE E. REEVES ET AL.: "The surf-4 gene encodes a novel 30kDa integral membrane protein" MOLECULAR MEMBRANE BIOLOGY, vol. 12, no. 2, April 1995 (1995-04), pages 201-208, XP002096695.

D2: CLARE HUXLEY ET AL.: "The mouse surfeit locus contains a cluster of six genes associated with four CpG-rich islands in 32 kilobases of genomic DNA" MOLECULAR AND CELLULAR BIOLOGY, vol. 10, no. 2, February 1990 (1990-02), pages 605-614, XP002096696 WASHINGTON US.

1. Documents D1 and D2 disclose the surf-4 gene, which encodes a 30kDa integral membrane protein sharing 100% sequence identity with Sequence No.1, as well as a vector comprising it and its production by Hela cells (see Experimental procedures in D1). Consequently, claims 1, 2, 5, and 6 are not novel and do not meet the requirements of Article 33(2) PCT.

2. Claims 3 and 4 relate to a cDNA comprising Sequences No.4 and 7. Since such cDNA has not been disclosed in the state of the art, claims 3 and 4 are novel and comply with the requirements of Article 33(2) PCT.

2.1. Document D1, which is considered to represent the most relevant state of the art, discloses the isolation of the coding region for the surf-4 gene. Document D1 differs only from the subject-matter of claims 3 and 4 in that the particular sequences Nos. 4 and 7 are not disclosed. However, the sequencing of an isolated gene is a routine procedure in the art, which the skilled person would perform without the need of exercising any inventive step. Consequently, claims 3 and 4 do not involve an inventive step and do not meet the requirements of Article 33(3) PCT.

Re Item VII

Certain defects in the international application

1. Claims 1, 3, and 4 contain references to the drawings. According to Rule 6.2(a) PCT, claims should not contain such references except where absolutely necessary, which is not the case here.

Re Item VIII

Certain observations on the international application

1. General statements in the description which imply that the extent of protection may be expanded in a not precisely defined way are not allowable. The inclusion of the

expression "hereby incorporated by reference" in the description contradicts therefore the requirements of Article 6.

2. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 and D2 is not mentioned in the description, nor are these documents identified therein.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, C07K 14/47, C12N 15/85, 5/10

A2

(11) International Publication Number: WO 99/27094

(43) International Publication Date: 3 June 1999 (03.06.99)

(21) International Application Number: PCT/JP98/05238

(22) International Filing Date: 20 November 1998 (20.11.98)

(30) Priority Data:
9/323129 25 November 1997 (25.11.97) JP

(71) Applicants *(for all designated States except US)*: SAGAMI CHEMICAL RESEARCH CENTER [JP/JP]; 4–1, Nishi–Ohnuma 4–chome, Sagamihara–shi, Kanagawa 229–0012 (JP). PROTEGENE INC. [JP/JP]; 2–20–3, Naka–cho, Meguro–ku, Tokyo 153–0065 (JP).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: KATO, Seishi [JP/JP]; 3–46–50, Wakamatsu, Sagamihara–shi, Kanagawa 229–0014 (JP). KIMURA, Tomoko [JP/JP]; 302, 4–1–28, Nishiikuta, Tama–ku, Kawasaki–shi, Kanagawa 214–0037 (JP). SEKINE, Shingo [JP/JP]; Remonzu 101, 2–8–15, Atago, Ageo–shi, Saitama 362–0034 (JP).

(74) Agents: AOYAMA, Tamotsu et al.; Aoyama & Partners, IMP Building, 3–7, Shiromi 1–chome, Chuo–ku, Osaka–shi, Osaka 540–0001 (JP).

(81) Designated States: AU, CA, JP, MX, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: HUMAN PROTEINS HAVING TRANSMEMBRANE DOMAINS AND DNAs ENCODING THESE PROTEINS

(57) Abstract

Human proteins having transmembrane domains, cDNAs coding for these proteins, and expression vectors of said cDNAs as well as eucaryotic cells expressing said cDNAs are provided. The proteins exist in the cell membrane and are considered to control the proliferation and the differentiation of the cells. The proteins can thus be employed as pharmaceuticals such as carcinostatic agents relating to the control of the proliferation and the differentiation of the cells or as antigens for preparing antibodies against said proteins. The cDNAs can be utilized as probes for the gene diagnosis and gene sources for the gene therapy. Furthermore, the cDNAs can be utilized for large–scale expression of said proteins. Cells, wherein these membrane protein genes are introduced and membrane proteins are expressed in large amounts, can be utilized for detection of the corresponding ligands, screening of novel low–molecular pharmaceuticals, and so on.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

DESCRIPTION

Human Proteins Having Transmembrane Domains and DNAs Encoding these Proteins

TECHNICAL FIELD

The present invention relates to human proteins having transmembrane domains, cDNAs coding for these proteins, and expression vectors of said cDNAs as well as eucaryotic cells expressing said cDNAs. The proteins of the present invention can be employed as pharmaceuticals or as antigens for preparing antibodies against said proteins. The human cDNAs of the present invention can be utilized as probes for the gene diagnosis and gene sources for the gene therapy. Furthermore, the cDNAs can be utilized as gene sources for large-scale production of the proteins encoded by said cDNAs. Cells, wherein these membrane protein genes are introduced and membrane proteins are expressed in large amounts, can be utilized for detection of the corresponding ligands, screening of novel low-molecular pharmaceuticals, and so on.

BACKGROUND ART

Membrane proteins play important roles, as signal receptors, ion channels, transporters, etc. in the material transportation and the information transmission which are mediated by the cell membrane. Examples thereof include receptors for a variety of cytokines, ion channels for the sodium ion, the potassium ion, the chloride ion, etc., transporters for saccharides and amino acids, and so on, where the genes of many of them have been cloned already.

It has been clarified that abnormalities of these membrane proteins are associated with a number of hitherto-cryptogenic diseases. For instance, a gene of a membrane protein having twelve transmembrane domains was identified as the gene responsible for cystic fibrosis [Rommens, J. M. et al., Science 245: 1059-1065 (1989)]. In addition, it has been clarified that several membrane proteins act as receptors when a virus infects the cells. For instance, HIV-1 is revealed to infect into the cells through mediation of a membrane protein fusin having a membrane protein on the T-cell membrane, a CD-4 antigen, and seven transmembrane domains [Feng, Y. et al., Science 272: 872-877 (1996)]. Therefore, discovery of a new membrane protein is anticipated to lead to elucidation of the causes of many diseases, so that isolation of a new gene coding for the membrane protein has been desired.

Heretofore, owing to difficulty in the purification, many membrane proteins have been isolated by an approach from the gene side. A general method is the so-called expression cloning which comprises transfection of a cDNA library in eucaryotic cells to express cDNAs and then detection of the cells expressing the target membrane protein on the membrane by an immunological technique using an antibody or a physiological technique on the change in the membrane permeability. However, this method is applicable only to cloning of a gene of a membrane protein with a known function.

In general, membrane proteins possess hydrophobic transmembrane domains inside the proteins, wherein, after synthesis thereof in the ribosome, these domains remain in

the phospholipid membrane to be trapped in the membrane. Accordingly, the evidence of the cDNA for encoding the membrane protein is provided by determination of the whole base sequence of a full-length cDNA followed by detection of highly hydrophobic transmembrane domains in the amino acid sequence of the protein encoded by said cDNA.

DISCLOSURE OF INVENTION

The object of the present invention is to provide novel human proteins having transmembrane domains, DNAs coding for said proteins, and expression vectors of said cDNAs as well as transformation eucaryotic cells that are capable of expressing said cDNAs.

As the result of intensive studies, the present inventors have been successful in cloning of cDNAs coding for proteins having transmembrane domains from the human full-length cDNA bank, thereby completing the present invention. In other words, the present invention provides human proteins having transmembrane domains, namely proteins containing any of the amino acid sequences represented by Sequence Nos. 1 to 3. Moreover, the present invention provides DNAs coding for the above-mentioned proteins, exemplified by cDNAs containing any of the base sequences represented by Sequence Nos. 4 to 6, 7, 9 and 11, as well as transformation eucaryotic cells that are capable of expressing said cDNAs.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP01207.

Figure 2: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP01862.

Figure 3: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10493.

BEST MODE FOR CARRYING OUT THE INVENTION

The proteins of the present invention can be obtained, for example, by a method for isolation from human organs, cell lines, etc., a method for preparation of peptides by the chemical synthesis, or a method for production with the recombinant DNA technology using the DNAs coding for the transmembrane domains of the present invention, wherein the method for obtainment by the recombinant DNA technology is employed preferably. For instance, in vitro expression of the proteins can be achieved by preparation of an RNA by in vitro transcription from a vector having one of cDNAs of the present invention, followed by in vitro translation using this RNA as a template. Also, recombination of the translation region into a suitable expression vector by the method known in the art leads to production of a large amount of the encoded protein by using prokaryotic cells such as *Escherichia coli*, *Bacillus subtilis*, etc., and eucaryotic cells such as yeasts, insect cells, mammalian cells, etc.

In the case in which a protein of the present invention is produced by expression of one of the DNAs by in vitro expression, recombination of the translation region in said cDNA into a vector having an RNA polymerase

promoter, followed by addition into an in vitro translation system such as a rabbit reticulocyte lysate, a wheat germ extract, or the like, allows in vitro production of the protein of the present invention. Examples of the RNA polymerase promoter include T7, T3, SP6, and so on. Vectors containing such an RNA polymerase promoter are exemplified by pKA1, pCDM8, pT3/T7 18, pT7/3 19, pBluescript II, and so on. Also, addition of the dog pancreas microsome etc. in the reaction system enables the membrane protein of the present invention to be expressed in a form integrated in the microsome membrane.

In the case in which a protein of the present invention is produced by expression of a DNA in a microorganism such as *Escherichia coli* etc., a recombinant expression vector bearing the translation region in the cDNA of the present invention is constructed in an expression vector having an origin, a promoter, a ribosome-binding site, a cDNA-cloning site, a terminator etc., which can be replicated in the microorganism, and, after transformation of the host cells with said expression vector, the thus-obtained transformant is incubated, whereby the protein encoded by said cDNA can be produced on a large scale in the microorganism. In this case, a protein fragment containing an optional region can be obtained by carrying out the expression with inserting an initiation codon and a termination codon in front of and behind an optional translation region. Alternatively, a fusion protein with another protein can be expressed. Only a protein portion coding for said cDNA can be obtained by cleavage of said fusion protein with a suitable protease.

Examples of the expression vector for *Escherichia coli* include the pUC system, pBluescript II, the pET expression system, the pGEX expression system, and so on.

In the case in which one of the proteins of the present invention is produced by expression of a DNA in eucaryotic cells, the protein of the present invention can be produced as a transmembrane protein on the cell-membrane surface, when the translation region of said cDNA is subjected to recombination to an expression vector for eucaryotic cells that has a promoter, a splicing region, a poly(A) insertion site, etc., followed by introduction into the eucaryotic cells. The expression vector is exemplified by pKA1, pED6dpc2, pCDM8, pSVK3, pMSG, pSVL, pBK-CMV, pBK-RSV, EBV vector, pRS, pYES2, and so on. Examples of eucaryotic cells to be used in general include mammalian culture cells such as simian kidney cells COS7, Chinese hamster ovary cells CHO, etc., budding yeasts, fission yeasts, silkworm cells, *Xenopus laevis* egg cells, and so on, but any eucaryotic cells may be used, provided that they are capable of expressing the present proteins on the membrane surface. The expression vector can be introduced in the eucaryotic cells by methods known in the art such as the electroporation method, the potassium phosphate method, the liposome method, the DEAE-dextran method, and so on.

After one of the proteins of the present invention is expressed in prokaryotic cells or eucaryotic cells, the objective protein can be isolated from the culture and purified by a combination of separation procedures known in the art. Such examples include treatment with a denaturing agent such as urea or a surface-active agent, sonication,

enzymatic digestion, salting-out or solvent precipitation, dialysis, centrifugation, ultrafiltration, gel filtration, SDS-PAGE, isoelectric focusing, ion-exchange chromatography, hydrophobic chromatography, affinity chromatography, reverse phase chromatography, and so on.

The proteins of the present invention include peptide fragments (more than 5 amino acid residues) containing any partial amino acid sequence in the amino acid sequences represented by Sequence Nos. 1 to 3. These peptide fragments can be utilized as antigens for preparation of antibodies. Hereupon, among the proteins of the present invention, those having the signal sequence are secreted in the form of maturation proteins on the surface of the cells, after the signal sequences are removed. Therefore, these maturation proteins shall come within the scope of the present invention. The N-terminal amino acid sequences of the maturation proteins can be easily identified by using the method for the cleavage-site determination in a signal sequence [Japanese Patent Kokai Publication No. 1996-187100]. Furthermore, some membrane proteins undergo the processing on the cell surface to be converted to the secretory forms. Such proteins or peptides in the secretory forms shall come within the scope of the present invention. When sugar chain-binding sites are present in the amino acid sequences, expression in appropriate eucaryotic cells affords proteins wherein sugar chains are added. Accordingly, such proteins or peptides wherein sugar chains are added shall come within the scope of the present invention.

The DNAs of the present invention include all DNAs

coding for the above-mentioned proteins. Said DNAs can be obtained by using a method by chemical synthesis, a method by cDNA cloning, and so on.

The cDNAs of the present invention can be cloned, for example, from cDNA libraries of the human cell origin. These cDNA are synthesized by using as templates poly(A)$^+$ RNAs extracted from human cells. The human cells may be cells delivered from the human body, for example, by the operation or may be the culture cells. The cDNAs can be synthesized by using any method selected from the Okayama-Berg method [Okayama, H. and Berg, P., Mol. Cell. Biol. 2: 161-170 (1982)], the Gubler-Hoffman method [Gubler, U. and Hoffman, J. Gene 25: 263-269 (1983)], and so on, but it is preferred to use the capping method [Kato, S. et al., Gene 150: 243-250 (1994)], as exemplified in Examples, in order to obtain a full-length clone in an effective manner. In addition, commercially available, human cDNA libraries can be utilized. Cloning of the cDNAs of the present invention from the cDNA libraries can be carried out by synthesis of an oligonucleotide on the basis of an optional portion in the cDNA base sequences of the present invention, followed by screening using this oligonucleotide as the probe according to the colony or plaque hybridization by a method known in the art. In addition, the cDNA fragments of the present invention can be prepared by synthesis of an oligonucleotide to be hybridized at both termini of the objective cDNA fragment, followed by the usage of this oligonucleotide as the primer for the RT-PCR method from an mRNA isolated from human cells.

The cDNAs of the present invention are characterized

by containing either of the base sequences represented by Sequence Nos. 4 to 6 or the base sequences represented by Sequence Nos. 7, 9 and 11. Table 1 summarizes the clone number (HP number), the cells affording the cDNA, the total base number of the cDNA, and the number of the amino acid residues of the encoded protein, for each of the cDNAs.

Table 1

Sequence No.	HP No.	Cell	Number of bases	Number of amino acids
1, 4, 7	HP01207	Stomach Cancer	2938	269
2, 5, 8	HP01862	Stomach Cancer	2290	311
3, 6, 9	HP10493	PMA-U937	3705	383

Hereupon, the same clones as the cDNAs of the present invention can be easily obtained by screening of the cDNA libraries constructed from the human cell lines and human tissues utilized in the present invention by the use of an oligonucleotide probe synthesized on the basis of the cDNA base sequence described in any of Sequence Nos. 4 to 6, 7, 9 and 11.

In general, the polymorphism due to the individual difference is frequently observed in human genes. Accordingly, any cDNA that is subjected to insertion or deletion of one or plural nucleotides and/or substitution with other nucleotides in Sequence Nos. 4 to 6, 7, 9 and 11 shall come within the scope of the present invention.

In a similar manner, any protein that is formed by these modifications comprising insertion or deletion of one

or plural amino acids and/or substitution with other amino acids shall come within the scope of the present invention, as far as the protein possesses the activity of any protein having the amino acid sequences represented by Sequence Nos. 1 to 3.

The cDNAs of the present invention include cDNA fragments (more than 10 bp) containing any partial base sequence in the base sequences represented by Sequence Nos. 4 to 6 or in the base sequences represented by Sequences No. 7, 9 and 11. Also, DNA fragments consisting of a sense chain and an anti-sense chain shall come within this scope. These DNA fragments can be utilized as the probes for the gene diagnosis.

In addition to the activities and uses described above, the polynucleotides and proteins of the present invention may exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

Research Uses and Utilities

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a

particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodiesusing DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used in assay to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in

biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E.F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

Nutritional Uses

Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In

such cases the protein or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

Cytokine and Cell Proliferation/Differentiation Activity

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing

Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., J. Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol. 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon γ, Schreiber, R.D. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6-Nordan, R. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and

Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark,S.C. and Turner, K.J. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

Immune Stimulating or Suppressing Activity

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders

(including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial orfungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present

invention.

Using the proteins of the invention it may also be possible to immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as , for example, B7)), e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune

reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins in vivo as described in Lenschow et al., Science 257:789-792 (1992) and Turka et

al., Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function in vivo on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the commoncold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the in vitro activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells in vivo.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity.

Tumor cells (e.g., sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected ex vivo with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection in vivo.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I α chain protein and β_2 microglobulin protein or an MHC class IIα chain protein and an MHC class IIβ chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a

peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowmanet al., J. Virology 61:1992-1998; Takai et al., J.

Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: In vitro antibody production, Mond, J.J. and Brunswick, M. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine

182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

Hematopoiesis Regulating Activity

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting

the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vivo or ex-vivo (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initiating cell assay, Sutherland, H.J. In Culture of Hematopoietic Cells. R.I.

Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

Tissue Growth Activity

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity,

etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors ex vivo for return in vivo to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as

a carrier as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, i.e. for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium),

muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, HI and Rovee, DT, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

Activin/Inhibin Activity

A protein of the present invention may also exhibit

activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin α family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin-β group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, United States Patent 4,798,885. A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

Chemotactic/Chemokinetic Activity

A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis)consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population

to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25: 1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153: 1762-1768, 1994.

Hemostatic and Thrombolytic Activity

A protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result,such a protein is expected to be useful in treatment of various coagulation disorders (includinghereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-

474, 1988.

Receptor/Ligand Activity

A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in:Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-

1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

Anti-Inflammatory Activity

Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of ytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

Tumor Inhibition Activity

In addition to the activities described above for immunological treatment or prevention of tumors, a protein

of the invention may exhibit other anti-tumor activities. A protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth

Other Activities

A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or caricadic cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive

disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

Examples

The present invention is embodied in more detail by the following examples, but this embodiment is not intended to restrict the present invention. The basic operations and the enzyme reactions with regard to the DNA recombination are carried out according to the literature ["Molecular Cloning. A Laboratory Manual " , Cold Spring Harbor Laboratory, 1989]. Unless otherwise stated, restrictive enzymes and a variety of modification enzymes to be used were those available from TAKARA SHUZO. The manufacturer's instructions were used for the buffer compositions as well as for the reaction conditions, in each of the enzyme reactions. The cDNA synthesis was carried out according to the literature [Kato, S. et al., Gene 150: 243-250 (1994)].

(1) Preparation of Poly(A)$^+$ RNA

The histiocyte lymphoma cell line U937 (ATCC CRL 1593) stimulated by phorbol ester and tissues of stomach cancer delivered by the operation were used for human cells to extract mRNAs. The cell line was incubated by a conventional procedure.

After about 1 g of the human cells was homogenized in 20 ml of a 5.5 M guanidinium thiocyanate solution, a total mRNA was prepared according to the literature [Okayama, H. et al., "Method in Enzymology", Vol. 164, Academic Press, 1987]. This was subjected to chromatography on oligo(dT)-cellulose column washed with a 20 mM Tris-hydrochloride buffer solution (pH 7.6), 0.5 M NaCl, and 1 mM EDTA to obtain a poly(A)$^+$ RNA according to the above-described literature.

(2) Construction of cDNA Library

Ten micrograms of the above-mentioned poly(A)$^+$ RNA were dissolved in a 100 mM Tris-hydrochloride buffer solution (pH 8), one unit of an RNase-free, bacterial alkaline phosphatase was added, and the reaction was run at 37 ℃ for one hour. After the reaction solution was subjected to phenol extraction, followed by ethanol precipitation, the resulting pellet was dissolved in a solution containing 50 mM sodium acetate (pH 6), 1 mM EDTA, 0.1% 2-mercaptoethanol, and 0.01% Triton X-100. Thereto was added one unit of a tobacco-origin acid pyrophosphatase (Epicentre Technologies) and a total 100 µl volume of the resulting mixture was reacted at 37℃ for one hour. After the reaction solution was subjected to phenol extraction, followed by ethanol precipitation, the resulting pellet was dissolved in water to obtain a solution of a decapped

poly(A)$^+$ RNA.

The decapped poly(A)$^+$ RNA and 3 nmol of a chimeric DNA-RNA oligonucleotide (5'-dG-dG-dG-dG-dA-dA-dT-dT-dC-dG-dA-G-G-A-3') were dissolved in a solution containing 50 mM Tris-hydrochloride buffer solution (pH 7.5), 0.5 mM ATP, 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, and 25% polyethylene glycol, whereto was added 50 units of T4RNA ligase and a total 30 µl volume of the resulting mixture was reacted at 20 ℃ for 12 hours. After the reaction solution was subjected to phenol extraction, followed by ethanol precipitation, the resulting pellet was dissolved in water to obtain a chimeric-oligo-capped poly(A)$^+$ RNA.

After digestion of vector pKA1 (Japanese Patent Kokai Publication No. 1992-117292) developed by the present inventors with KpnI, about 60 dT tails were added using a terminal transferase. A vector primer to be used below was prepared by digestion of this product with EcoRV to remove a dT tail at one side.

After 6 µg of the previously-prepared chimeric-oligo-capped poly(A)$^+$ RNA was annealed with 1.2 µg of the vector primer, the resulting product was dissolved in a solution containing 50 mM Tris-hydrochloride buffer solution (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, and 1.25 mM dNTP (dATP + dCTP + dGTP + dTTP), 200 units of a reverse transcriptase (GIBCO-BRL) were added, and the reaction in a total 20 µl volume was run at 42℃ for one hour. After the reaction solution was subjected to phenol extraction, followed by ethanol precipitation, the resulting pellet was dissolved in a solution containing 50 mM Tris-hydrochloride buffer solution (pH 7.5), 100 mM NaCl, 10 mM $MgCl_2$, and 1

mM dithiothreitol. Thereto were added 100 units of EcoRI and a total 20 µl volume of the resulting mixture was reacted at 37℃ for one hour. After the reaction solution was subjected to phenol extraction, followed by ethanol precipitation, the resulting pellet was dissolved in a solution containing 20 mM Tris-hydrochloride buffer solution (pH 7.5), 100 mM KCl, 4 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, and 50 µg/ml of the bovine serum albumin. Thereto were added 60 units of an *Escherichia coli* DNA ligase and the resulting mixture was reacted at 16℃ for 16 hours. To the reaction solution were added 2 µl of 2 mM dNTP, 4 units of *Escherichia coli* DNA polymerase I, and 0.1 unit of *Escherichia coli* RNase H and the resulting mixture was reacted at 12℃ for one hour and then at 22℃ for one hour.

Next, the cDNA-synthesis reaction solution was used for transformation of *Escherichia coli* DH12S (GIBCO-BRL). The transformation was carried out by the electroporation method. A portion of the transformant was sprayed on the 2xYT agar culture medium containing 100 µg/ml ampicillin and the mixture was incubated at 37℃ overnight. A colony formed on the agar medium was picked up at random and inoculated on 2 ml of the 2xYT culture medium containing 100 µg/ml ampicillin. After incubation at 37℃ overnight, the culture mixture was centrifuged to separate the mycelia, from which a plasmid DNA was prepared by the alkaline lysis method. The plasmid DNA was subjected to double digestion with EcoRI and NotI, followed by 0.8% agarose gel electrophoresis, to determine the size of the cDNA insert. Furthermore, using the thus-obtained plasmid as a template, the sequence reaction was carried out by using an M13

universal primer labeled with a fluorescent dye and a Taq polymerase (a kit of Applied Biosystems) and then the product was examined with a fluorescent DNA sequencer (Applied Biosystems) to determine an about 400-bp base sequence at the 5'-terminus of the cDNA. The sequence data were filed as the homo/protein cDNA bank database.

(3) Selection of cDNAs Encoding Proteins Having Transmembrane Domains

A base sequence registered in the homo/protein cDNA bank was converted to three frames of amino acid sequences and the presence or absence of an open reading frame (ORF) beginning from the initiation codon was examined. Then, the selection was made for the presence of a signal sequence that is characteristic to a secretory protein at the N-terminus of the portion encoded by the ORF. These clones were sequenced from the both 5' and 3' directions by the use of the deletion method using exonuclease III to determine the whole base sequence. The hydrophobicity/hydrophilicity profiles were obtained for proteins encoded by the ORF by the Kyte-Doolittle method [Kyte, J. & Doolittle, R. F., J. Mol. Biol. 157: 105-132 (1982)] to examine the presence or absence of a hydrophobic region. In the case in which there is a hydrophobic region of a putative transmembrane domain in the amino acid sequence of an encoded protein, this protein was judged as a membrane protein.

(4) Functional Verification of Secretory Signal Sequence or Transmembrane Domains

It was verified by the method described in the literature [Yokoyama-Kobayashi, M. et al., Gene 163: 193-

196 (1995)] that the N-terminal hydrophobic region in the secretory protein clone candidate obtained in the above-mentioned steps functions as a secretory signal sequence. First, the plasmid containing the target cDNA was cleaved at an appropriate restriction enzyme site existing at the downstream of the portion expected for encoding the secretory signal sequence. In the case in which this restriction site was a protruding terminus, the site was blunt-ended by the Klenow treatment or treatment with the mung-bean nuclease. Digestion with HindIII was further carried out and a DNA fragment containing the SV40 promoter and a cDNA encoding the secretory signal sequence at the downstream of the promoter was separated by agarose gel electrophoresis. The resulting fragment was inserted between HindIII in pSSD3 (DDBJ/EMBL/GenBank Registration No. AB007632) and a restriction enzyme site selected so as to match with the urokinase-coding frame, thereby constructing a vector expressing a fusion protein of the secretory signal sequence of the target cDNA and the urokinase protease domain.

After *Escherichia coli* (host: JM109) bearing the fusion-protein expression vector was incubated at 37℃ for 2 hours in 2 ml of the 2xYT culture medium containing 100 μg/ml of ampicillin, the helper phage M13KO7 (50 μl) was added and the incubation was continued at 37℃ overnight. A supernatant separated by centrifugation underwent precipitation with polyethylene glycol to obtain single-stranded phage particles. These particles were suspended in 100 μl of 1 mM Tris-0.1 mM EDTA, pH 8 (TE). Also, there were used as controls suspensions of single-stranded phage

particles prepared in the same manner from pSSD3 and from the vector pKA1-UPA containing a full-length cDNA of urokinase [Yokoyama-Kobayashi, M. et al., Gene 163: 193-196 (1995)].

The culture cells originating from the simian kidney, COS7, were incubated at 37℃ in the presence of 5% CO_2 in the Dulbecco's modified Eagle's culture medium (DMEM) containing 10% fetal calf albumin. Into a 6-well plate (Nunc Inc., 3 cm in the well diameter) were inoculated 1×10^5 COS7 cells and incubation was carried out at 37℃ for 22 hours in the presence of 5% CO_2. After the culture medium was removed, the cell surface was washed with a phosphate buffer solution and then washed again with DMEM containing 50 mM Tris-hydrochloric acid (pH 7.5) (TDMEM). To the resulting cells was added a suspension of 1 μl of the single-stranded phage suspension, 0.6 ml of the DMEM culture medium, and 3 μl of TRANSFECTAM™ (IBF Inc.) and the resulting mixture was incubated at 37℃ for 3 hours in the presence of 5% CO_2. After the sample solution was removed, the cell surface was washed with TDMEM, 2 ml per well of DMEM containing 10% fetal calf albumin was added, and the incubation was carried out at 37℃ for 2 days in the presence of 5% CO_2.

To 10 ml of a 50 mM phosphate buffer solution (pH 7.4) containing 2% bovine fibrinogen (Miles Inc.), 0.5% agarose, and 1 mM calcium chloride were added 10 units of human thrombin (Mochida Pharmaceutical Co., Ltd.) and the resulting mixture was solidified in a plate of 9 cm in diameter to prepare a fibrin plate. Ten microliters of the culture supernatant of the tansfected COS7 cells were

spotted on the fibrin plate, which was incubated at 37℃ for 15 hours. In the case in which a clear circle appears on the fibrin plate, it is judged that the cDNA fragment codes for the amino acid sequence functioning as a secretory signal sequence. On the other hand, in case in which a clear circle is not formed, the cells were washed well, then the fibrin sheet was placed on the cells, and incubation was carried out at 37℃ for 15 hours. In case in which a clear portion is formed on the fibrin sheet, it indicates that the urokinase activity was expressed on the cell surface. In other words, the cDNA fragment is judged to code for the transmembrane domains.

(5) Protein Synthesis by In Vitro Translation

The plasmid vector bearing the cDNA of the present invention was used for in vitro transcription/translation with a T_NT rabbit reticulocyte lysate kit (Promega). In this case, [^{35}S]methionine was added to label the expression product with a radioisotope. Each of the reactions was carried out according to the protocols attached to the kit. Two micrograms of the plasmid was reacted at 30℃ for 90 minutes in a total 25 μl volume of the reaction solution containing 12.5 μl of T_NT rabbit reticulocyte lysate, 0.5 μl of a buffer solution (attached to kit), 2 μl of an amino acid mixture (methionine-free), 2 μl of [^{35}S]methionine (Amersham) (0.37 MBq/μl), 0.5 μl of T7RNA polymerase, and 20 U of RNasin. To 3 μl of the resulting reaction solution was added 2 μl of the SDS sampling buffer (125 mM Tris-hydrochloric acid buffer, pH 6.8, 120 mM 2-mercaptoethanol, 2% SDS solution, 0.025% bromophenol blue, and 20% glycerol) and the resulting

mixture was heated at 95℃ for 3 minutes and then subjected to SDS-polyacrylamide gel electrophoresis. The molecular weight of the translation product was determined by carrying out the autoradiograph.

(6) Expression by COS7

Escherichia coli bearing the expression vector of the protein of the present invention was infected with helper phage M13KO7 and single-stranded phage particles were obtained by the above-mentioned procedure. The thus-obtained phage was used for introducing each expression vector in the culture cells originating from the simian kidney, COS7. After incubation at 37℃ for 2 days in the presence of 5% CO_2, the incubation was continued for one hour in the culture medium containing [^{35}S]cystine or [^{35}S]methionine. Collection and dissolution of the cells, followed by subjecting to SDS-PAGE, allowed to observe the presence of a band corresponding to the expression product of each protein, which did not exist in the COS7 cells. For instance, HP01207 produced a band of 25 kDa in the membrane fraction.

(7) Clone Examples

<HP01207> (Sequence Nos. 1, 4, and 7)

Determination of the whole base sequence of the cDNA insert of clone HP01207 obtained from cDNA libraries of human stomach cancer revealed the structure consisting of a 100-bp 5'-nontranslation region, an 810-bp ORF, and a 2028-bp 3'-nontranslation region. The ORF codes for a protein consisting of 269 amino acid residues and there existed seven transmembrane domains. Figure 1 depicts the hydrophobicity/hydrophilicity profile, obtained by the

Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a smear translation product of a high molecular weight.

The search of the protein data base by using the amino acid sequence of the present protein revealed that the protein was analogous to the mouse Surf-4 protein (PIR Accession No. A34727). Table 2 shows the comparison of the amino acid sequence between the human protein of the present invention (HP) and the mouse Surf-4 protein (MM). Therein, the marks of * and . represent an amino acid residue identical with the protein of the present invention and an amino acid residue analogous to the protein of the present invention, respectively. The both proteins possessed a homology of 99.3% in the entire region.

Table 2

```
HS MGQNDLMGTAEDFADQFLRVTKQYLPHVARLCLISTFLEDGIRMWFQWSEQRDYIDTTWN
   ************************************************************.
MM MGQNDLMGTAEDFADQFLRVTKQYLPHVARLCLISTFLEDGIRMWFQWSEQRDYIDTTWS
HS CGYLLASSFVFLNLLGQLTGCVLVLSRNFVQYACFGLFGIIALQTIAYSILWDLKFLMRN
   ************************************************************
MM CGYLLASSFVFLNLLGQLTGCVLVLSRNFVQYACFGLFGIIALQTIAYSILWDLKFLMRN
HS LALGGGLLLLLAESRSEGKSMFAGVPTMRESSPKQYMQLGGRVLLVLMFMTLLHFDASFF
   ************************************************************
MM LALGGGLLLLLAESRSEGKSMFAGVPTMRESSPKQYMQLGGRVLLVLMFMTLLHFDASFF
HS SIVQNIVGTALMILVAIGFKTKLAALTLVVWLFAINVYFNAFWTIPVYKPMHDFLKYDFF
   **.********************************************************
MM SIIQNIVGTALMILVAIGFKTKLAALTLVVWLFAINVYFNAFWTIPVYKPMHDFLKYDFF
HS QTMSVIGGLLLVVALGPGGVSMDEKKKEW
   ****************************
MM QTMSVIGGLLLVVALGPGGVSMDEKKKEW
```

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of a base sequence that exhibited an analogy of 98.6% with a 762-bp part from position 122 up to position 883 (GenBank Accession No. Y14820), which codes for the fragment of the present protein.

The mouse Surf-4 protein is one of proteins which are encoded in the mouse surfeit locus and has been considered to a housekeeping protein that is essential to the survival of cells [Huxley, C. et al., Mol. Cell. Biol. 10: 605-614 (1990)].

<HP01862> (Sequence Nos. 2, 5 and 9)

Determination of the whole base sequence of the cDNA insert of clone HP01862 obtained from cDNA libraries of human stomach cancer revealed the structure consisting of an 80-bp 5'-nontranslation region, a 936-bp ORF, and a 1274-bp 3'-nontranslation region. The ORF codes for a protein consisting of 311 amino acid residues and there existed seven transmembrane domains. Figure 2 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a smear translation product of a high molecular weight.

The search of the protein data base using the amino acid sequence of the present protein has revealed the presence of sequences that were analogous to the rat NMDA receptor glutamate-binding subunit (GenBank Accession No. S19586). Table 3 shows the comparison of the amino acid sequence between the human protein of the present invention (HP) and the rat NMDA receptor glutamate-binding subunit

(RN). Therein, the marks of -, *, and . represent a gap, an amino acid residue identical with the protein of the present invention, and an amino acid residue analogous to the protein of the present invention, respectively. The both proteins possessed a homology of 41.0%.

Table 3

```
HS                                                  MSNPSAPPPYEDRNP

RN MKRVSWSLGTAILPQTLAILWGHKPLCLPMFSLPTLGPHTHRPLSSPLPMVNQGIPMVPV
HS LYPGPLPPGGYGQPSVLPGGYPAYPGYPQPGYGHPAGYPQPMPPTHPMPMNYGPGHGYDG
         **  .  . .. .* **. * .*.  **.*. ...* .* *   . **  **.
RN PITRWLPLKDLLKEATHQGHYPQSP-FPPNPYGQPPPFQDPGSPQHGNYQEEGPPSYYDN
HS EERAVSDSFGPGEWDDRKVRHTFIRKVYSIISVQLLITVAIIAIFTFVEPVSAFVRRNVA
   ..       * . .* *...*..*****. ....** .*....******. *..*** **
RN QD------FPSVNW-DKSIRQAFIRKVFLVLTLQLSVTLSTVAIFTFVGEVKGFVRANVW
HS VYYVSYAVFVVTYLILACCQGPRRRFPWNIILLTLFTFAMGFMTGTISSMYQTKAVIIAM
   .******.* .. ..*.** . *.. ***.. *...*.....*.* *.*.*.*.***.*.
RN TYYVSYAIFFISLIVLSCCGDFRKKHPWNLVALSILTISLSYMVGMIASFYNTEAVIMAV
HS IITAVVSISVTIFCFQTKVDFTSCTGLFCVLGIVLLVTGIVTSIVLYFQYVYWLHMLYAA
    **..*...*.**..**. ***** *.. *  .**.. .*.    ....    *...**.
RN GITTAVCFTVVIFSMQTRYDFTSCMGVLLVSVVVLFIFAIL---CIFIRNRI-LEIVYAS
HS LGAICFTLFLAYDTQLVLGNRKHTISPEDYITGALQIYTDIIYIFTFVLQLMGDRN
   ***. ** *** ****.***.. ..***.*. .**..***** ** ..* ..*
RN LGALLFTCFLAVDTQLLLGNKQLSLSPEEYVFAALNLYTDIINIFLYILTIIGRSQGIGQ
```

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the presence of sequences that possessed a homology of 90% or more (for example, Accession No. H06014) in EST, but any of the sequences was shorter than the present cDNAs and was not

found to contain the initiation codon.

The rat NMDA receptor glutamate-binding subunit is one of subunits of an NMDA receptor complex which exist specifically in the brain [Kumar, K. N. et al., Nature 354: 70-73 (1991)]. The protein of the present invention has seven transmembrane domains characteristic to channels and transporters and thereby is considered to play an important role as a channel and a transporter.

<HP10493> (Sequence Nos. 3, 6 and 11)

Determination of the whole base sequence of the cDNA insert of clone HP10493 obtained from cDNA libraries of the human lymphoma U937 revealed the structure consisting of a 123-bp 5'-nontranslation region, a 1152-bp ORF, and a 2430-bp 3'-nontranslation region. The ORF codes for a protein consisting of 383 amino acid residues and there existed one transmembrane domain at the N-terminus. Figure 3 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. Introduction of an expression vector, wherein the HindIII-AccI fragment containing a cDNA portion coding for the N-terminal 44 amino acid residues of the present protein was inserted into the HindIII-PmaCI site of pSSD3, into the COS7 cells revealed the urokinase activity on the cell surface to indicate that the present protein is the type-II membrane protein. In vitro translation resulted in formation of a translation product of 43 kDa that was almost consistent with the molecular weight of 43,001 predicted from the ORF.

The search of the protein data base using the amino acid sequence of the present protein has not revealed the presence of any known protein having an analogy. The search

of the motif sequences has revealed a high probability that histidine at position 175 is an active site of the trypsin-type serine protease. Accordingly, the present protein is likely to be a membrane-type protease. Also, the GenBank using the base sequences of the present cDNA has revealed the presence of sequences that possessed a homology of 90% or more (for example, Accession No. R81003) in EST, but many sequences were not distinct and the same ORF as that in the present cDNA was not found.

INDUSTRIAL APPLICABILITY

The present invention provides human proteins having transmembrane domains, cDNAs coding for these proteins, and expression vectors of said cDNAs as well as eucaryotic cells expressing said cDNAs. All of the proteins of the present invention exist in the cell membrane, so that they are considered to be proteins controlling the proliferation and the differentiation of the cells. Accordingly, the proteins of the present invention can be employed as pharmaceuticals such as carcinostatic agents relating to the control of the proliferation and the differentiation of the cells or as antigens for preparing antibodies against said proteins. The cDNAs of the present invention can be utilized as probes for the gene diagnosis and gene sources for the gene therapy. Furthermore, the cDNAs can be utilized for large-scale expression of said proteins. Cells, wherein these membrane protein genes are introduced and membrane proteins are expressed in large amounts, can be utilized for detection of the corresponding ligands, screening of novel low-molecular pharmaceuticals, and so on.

The present invention also provides genes corresponding to the polynucleotide sequences disclosed herein. "Corresponding genes" are the regions of the genome that are transcribed to produce the mRNAs from which cDNA polynucleotide sequences are derived and may include contiguous regions of the genome necessary for the regulated expression of such genes. Corresponding genes may therefore include but are not limited to coding sequences, 5' and 3' untranslated regions, alternatively spliced exons, introns, promoters, enhancers, and silencer or suppressor elements. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. An "isolated gene" is a gene that has been separated from the adjacent coding sequences, if any, present in the genome of the organism from which the gene was isolated.

Organisms that have enhanced, reduced, or modified expression of the gene(s) corresponding to the polynucleotide sequences disclosed herein are provided. The desired change in gene expression can be achieved through the use of antisense polynucleotides or ribozymes that bind and/or cleave the mRNA transcribed from the gene (Albert and Morris, 1994, Trends Pharmacol. Sci. 15(7): 250-254; Lavarosky et al., 1997, Biochem. Mol. Med. 62(1): 11-22; and Hampel, 1998, Prog. Nucleic Acid Res. Mol. Biol.

58: 1-39; all of which are incorporated by reference herein). Transgenic animals that have multiple copies of the gene(s) corresponding to the polynucleotide sequences disclosed herein, preferably produced by transformation of cells with genetic constructs that are stably maintained within the transformed cells and their progeny, are provided. Transgenic animals that have modified genetic control regions that increase or reduce gene expression levels, or that change temporal or spatial patterns of gene expression, are also provided (see European Patent No. 0 649 464 B1, incorporated by reference herein). In addition, organisms are provided in which the gene(s) corresponding to the polynucleotide sequences disclosed herein have been partially or completely inactivated, through insertion of extraneous sequences into the corresponding gene(s) or through deletion of all or part of the corresponding gene(s). Partial or complete gene inactivation can be accomplished through insertion, preferably followed by imprecise excision, of transposable elements (Plasterk, 1992, Bioessays 14(9): 629-633; Zwaal et al., 1993, Proc. Natl. Acad. Sci. USA 90(16): 7431-7435; Clark et al., 1994, Proc. Natl. Acad. Sci. USA 91(2): 719-722; all of which are incorporated by reference herein), or through homologous recombination, preferably detected by positive/negative genetic selection strategies (Mansour et al., 1988, Nature 336: 348-352; U.S. Patent Nos. 5,464,764; 5,487,992; 5,627,059; 5,631,153; 5,614, 396; 5,616,491; and 5,679,523; all of which are incorporated by reference herein). These organisms with altered gene expression are preferably eukaryotes and more preferably are mammals. Such organisms

are useful for the development of non-human models for the study of disorders involving the corresponding gene(s), and for the development of assay systems for the identification of molecules that interact with the protein product(s) of the corresponding gene(s).

Where the protein of the present invention is membrane-bound (e.g., is a receptor), the present invention also provides for soluble forms of such protein. In such forms part or all of the intracellular and transmembrane domains of the protein are deleted such that the protein is fully secreted from the cell in which it is expressed. The intracellular and transmembrane domains of proteins of the invention can be identified in accordance with known techniques for determination of such domains from sequence information.

Proteins and protein fragments of the present invention include proteins with amino acid sequence lengths that are at least 25%(more preferably at least 50%, and most preferably at least 75%) of the length of a disclosed protein and have at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with that disclosed protein, where sequence identity is determined by comparing the amino acid sequences of the proteins when aligned so as to maximize overlap and identity while minimizing sequence gaps. Also included in the present invention are proteins and protein fragments that contain a segment preferably comprising 8 or more (more preferably 20 or more, most preferably 30 or more) contiguous amino acids that shares at least 75% sequence identity (more preferably, at least 85% identity;

most preferably at least 95% identity) with any such segment of any of the disclosed proteins.

Species homologs of the disclosed polynucleotides and proteins are also provided by the present invention. As used herein, a "species homologue" is a protein or polynucleotide with a different species of origin from that of a given protein or polynucleotide, but with significant sequence similarity to the given protein or polynucleotide, as determined by those of skill in the art. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species.

The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotide which also encode proteins which are identical, homologous, or related to that encoded by the polynucleotides.

The invention also includes polynucleotides with sequences complementary to those of the polynucleotides disclosed herein.

The present invention also includes polynucleotides capable of hybridizing under reduced stringency conditions, more preferably stringent conditions, and most preferably highly stringent conditions, to polynucleotides described herein. Examples of stringency conditions are shown in the table below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for

example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

Table

Stringency Condition	Polynucleotide Hybrid	Hybrid Length (bp)‡	Hybridization Temperature and Buffer†	Wash Temperature and Buffer†
A	DNA : DNA	≥50	65°C; 1×SSC -or- 42°C; 1×SSC,50% formamide	65°C; 0.3×SSC
B	DNA : DNA	<50	T_B*; 1×SSC	T_B*; 1×SSC
C	DNA : RNA	≥50	67°C; 1×SSC -or- 45°C; 1×SSC,50% formamide	67°C; 0.3×SSC
D	DNA : RNA	<50	T_D*; 1×SSC	T_D*; 1×SSC
E	RNA : RNA	≥50	70°C; 1×SSC -or- 50°C; 1×SSC,50% formamide	70°C; 0.3×SSC
F	RNA : RNA	<50	T_F*; 1×SSC	T_F*; 1×SSC
G	DNA : DNA	≥50	65°C; 4×SSC -or- 42°C; 4×SSC,50% formamide	65°C; 1×SSC
H	DNA : DNA	<50	T_H*; 4×SSC	T_H*; 4×SSC
I	DNA : RNA	≥50	67°C; 4×SSC -or- 45°C; 4×SSC,50% formamide	67°C; 1×SSC
J	DNA : RNA	<50	T_J*; 4×SSC	T_J*; 4×SSC
K	RNA : RNA	≥50	70°C; 4×SSC -or- 50°C; 4×SSC,50% formamide	67°C; 1×SSC
L	RNA : RNA	<50	T_L*; 2×SSC	T_L*; 2×SSC
M	DNA : DNA	≥50	50°C; 4×SSC -or- 40°C; 6×SSC,50% formamide	50°C; 2×SSC
N	DNA : DNA	<50	T_N*; 6×SSC	T_N*; 6×SSC
O	DNA : RNA	≥50	55°C; 4×SSC -or- 42°C; 6×SSC,50% formamide	55°C; 2×SSC
P	DNA : RNA	<50	T_P*; 6×SSC	T_P*; 6×SSC
Q	RNA : RNA	≥50	60°C; 4×SSC -or- 45°C; 6×SSC,50% formamide	60°C; 2×SSC
R	RNA : RNA	<50	T_R*; 4×SSC	T_R*; 4×SSC

‡ : The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.

† : SSPE (1×SSPE is 0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH7.4) can be substituted for SSC (1×SSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete.

*T_B - T_R : The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, T_m(°C)=2(#of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49 base pairs in length, T_m(°C)=81.5 + 16.6($\log_{10}$[Na^+]) + 0.41 (%G+C) - (600/N), where N is the number of bases in the hybrid, and [Na^+] is the concentration of sodium ions in the hybridization buffer ([Na^+] for 1×SSC=0.165M).

Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, chapters 9 and 11, and Current Protocols in Molecular Biology, 1995, F.M. Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4, incorporated herein by reference.

Preferably, each such hybridizing polynucleotide has a length that is at least 25% (more preferably at least 50%, and most preferably at least 75%) of the length of the polynucleotide of the present invention to which it hybridizes, and has at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with the polynucleotide of the present

invention to which it hybridizes, where sequence identity is determined by comparing the sequences of the hybridizing polynucleotides when aligned so as to maximize overlap and identity while minimizing sequence gaps.

CLAIMS

1. A protein comprising any one of the amino acid sequences represented by Sequence Nos. 1 to 3.

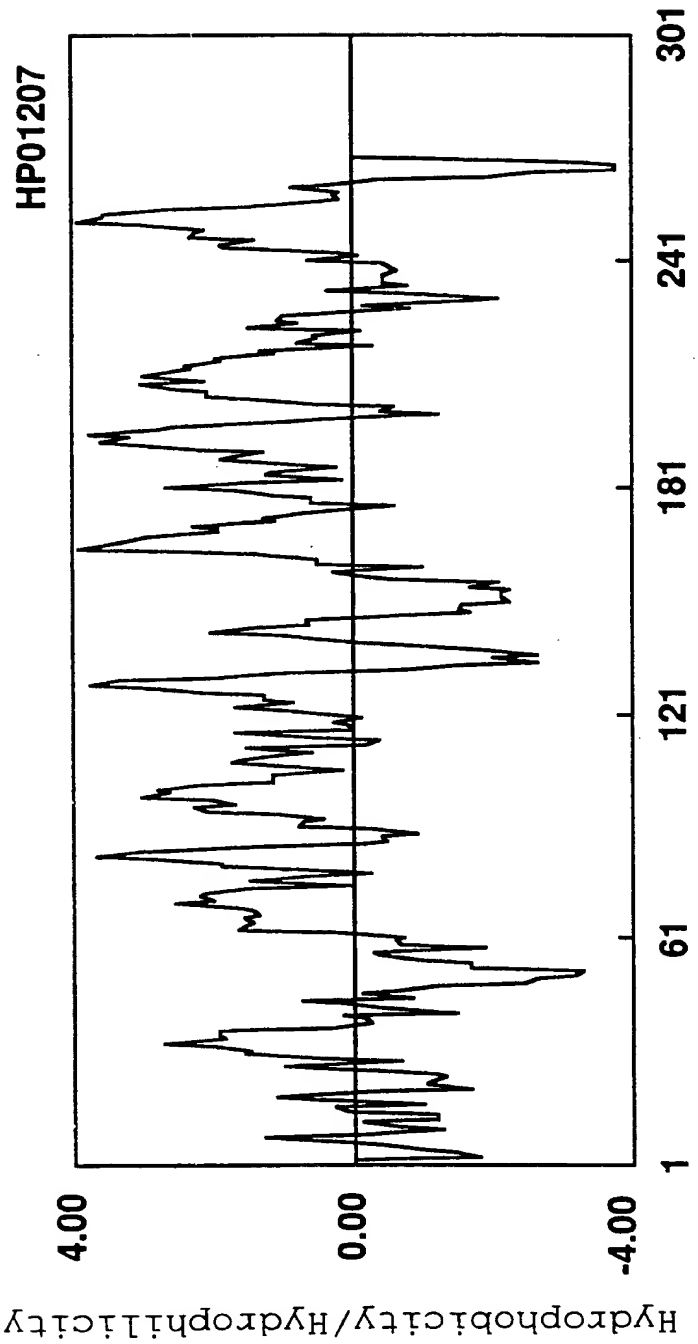
2. A DNA coding for the protein according to Claim 1.

3. A cDNA comprising any one of the base sequences represented by Sequence Nos. 4 to 6.

4. The cDNA according to Claim 3 comprising any one of the base sequences represented by Sequence Nos. 7, 9 and 11.

5. An expression vector capable of expressing the DNA according to any one of Claims 2 to 4 by in vitro translation or in eucaryotic cells.

6. A transformation eucaryotic cell capable of expressing the DNA according to any one of Claims 2 to 4 and of producing the protein according to Claim 1.


HP01207
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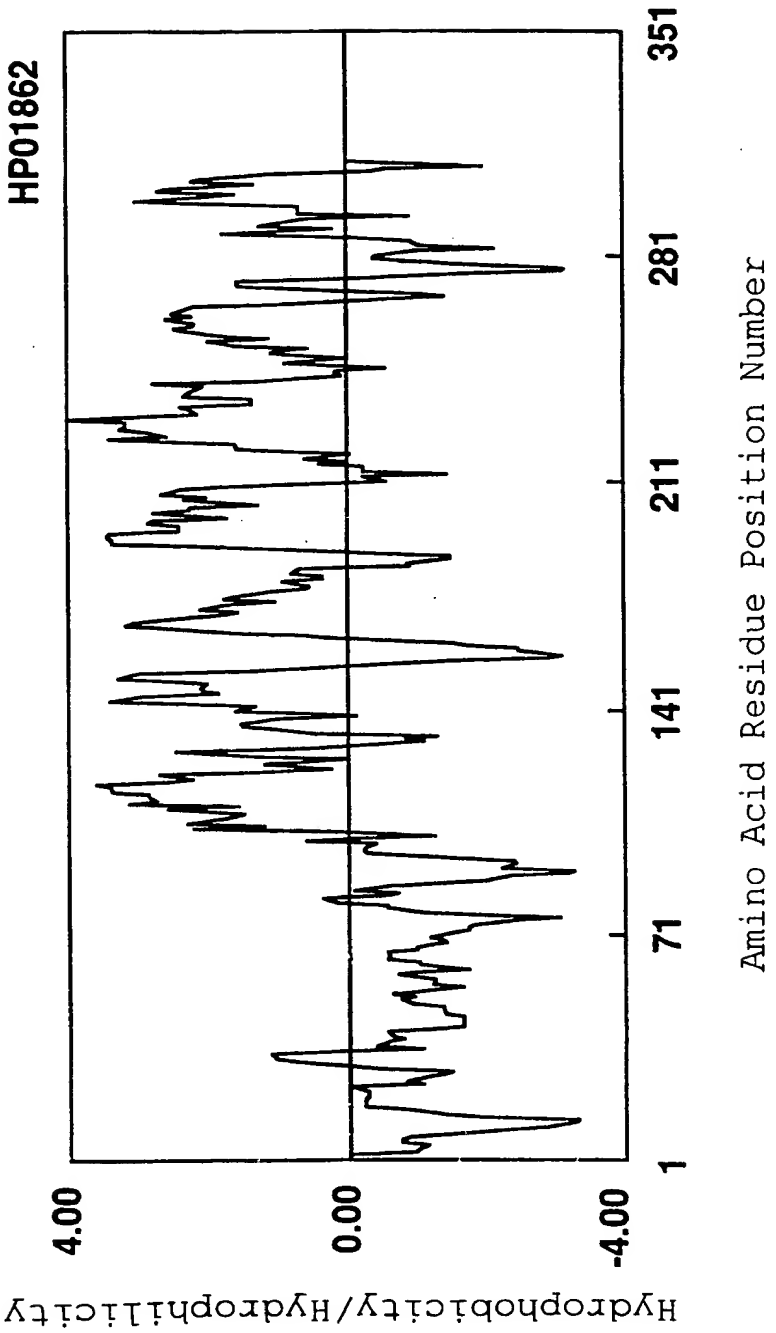
-4.00
1
61
121
181
241
301
Hydrophobicity/Hydrophilicity
Amino Acid Residue Position Number

Fig.1


HP01862
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-4.00
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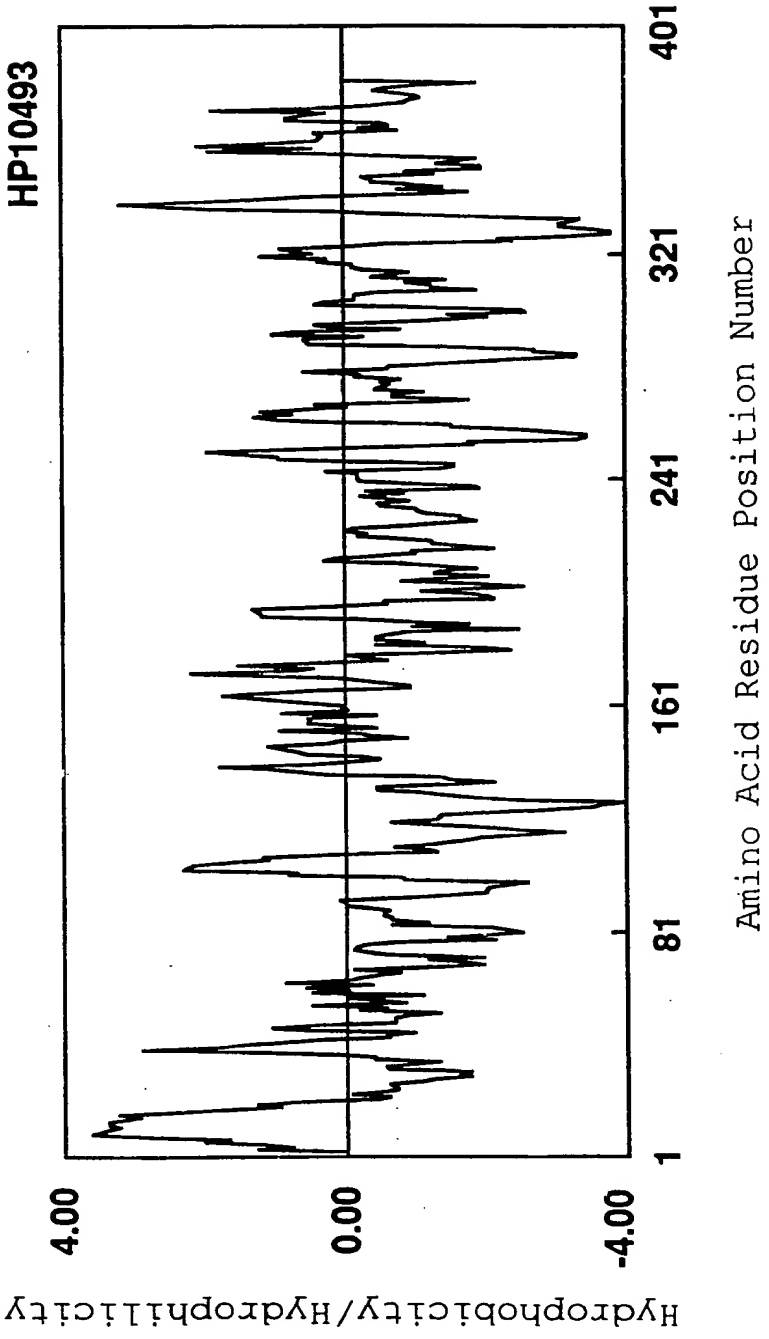
71
141
211
281
351
Hydrophobicity/Hydrophilicity
Amino Acid Residue Position Number

Fig.2


HP10493
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-4.00
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161
241
321
401
Hydrophobicity/Hydrophilicity
Amino Acid Residue Position Number

Fig.3

SEQUENCE LISTING

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<140>

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<150> Japan 9-323129

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<210> 1

<211> 269

<212> PRT

<213> Homo sapiens

<400> 1

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20 25 30

Leu Ile Ser Thr Phe Leu Glu Asp Gly Ile Arg Met Trp Phe Gln Trp

35 40 45

Ser Glu Gln Arg Asp Tyr Ile Asp Thr Thr Trp Asn Cys Gly Tyr Leu

50 55 60

Leu Ala Ser Ser Phe Val Phe Leu Asn Leu Leu Gly Gln Leu Thr Gly

65 70 75 80

Cys Val Leu Val Leu Ser Arg Asn Phe Val Gln Tyr Ala Cys Phe Gly

85 90 95

Leu Phe Gly Ile Ile Ala Leu Gln Thr Ile Ala Tyr Ser Ile Leu Trp

100 105 110

Asp Leu Lys Phe Leu Met Arg Asn Leu Ala Leu Gly Gly Gly Leu Leu

115 120 125

Leu Leu Leu Ala Glu Ser Arg Ser Glu Gly Lys Ser Met Phe Ala Gly

130 135 140

Val Pro Thr Met Arg Glu Ser Ser Pro Lys Gln Tyr Met Gln Leu Gly

145 150 155 160

Gly Arg Val Leu Leu Val Leu Met Phe Met Thr Leu Leu His Phe Asp

165 170 175

Ala Ser Phe Phe Ser Ile Val Gln Asn Ile Val Gly Thr Ala Leu Met

180 185 190

Ile Leu Val Ala Ile Gly Phe Lys Thr Lys Leu Ala Ala Leu Thr Leu

195 200 205

Val Val Trp Leu Phe Ala Ile Asn Val Tyr Phe Asn Ala Phe Trp Thr

210 215 220

Ile Pro Val Tyr Lys Pro Met His Asp Phe Leu Lys Tyr Asp Phe Phe

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20 25 30

Pro Gly Gly Tyr Pro Ala Tyr Pro Gly Tyr Pro Gln Pro Gly Tyr Gly
35 40 45

His Pro Ala Gly Tyr Pro Gln Pro Met Pro Pro Thr His Pro Met Pro
50 55 60

Met Asn Tyr Gly Pro Gly His Gly Tyr Asp Gly Glu Glu Arg Ala Val
65 70 75 80

Ser Asp Ser Phe Gly Pro Gly Glu Trp Asp Asp Arg Lys Val Arg His
85 90 95

Thr Phe Ile Arg Lys Val Tyr Ser Ile Ile Ser Val Gln Leu Leu Ile
100 105 110

Thr Val Ala Ile Ile Ala Ile Phe Thr Phe Val Glu Pro Val Ser Ala
115 120 125

Phe Val Arg Arg Asn Val Ala Val Tyr Tyr Val Ser Tyr Ala Val Phe
130 135 140
Val Val Thr Tyr Leu Ile Leu Ala Cys Cys Gln Gly Pro Arg Arg Arg
145 150 155 160
Phe Pro Trp Asn Ile Ile Leu Leu Thr Leu Phe Thr Phe Ala Met Gly
165 170 175
Phe Met Thr Gly Thr Ile Ser Ser Met Tyr Gln Thr Lys Ala Val Ile
180 185 190
Ile Ala Met Ile Ile Thr Ala Val Val Ser Ile Ser Val Thr Ile Phe
195 200 205
Cys Phe Gln Thr Lys Val Asp Phe Thr Ser Cys Thr Gly Leu Phe Cys
210 215 220
Val Leu Gly Ile Val Leu Leu Val Thr Gly Ile Val Thr Ser Ile Val
225 230 235 240
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245 250 255
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            20                  25                  30
Pro Ala Tyr Arg Leu Pro Val Val Leu Pro Gln Ser Thr Leu Asn Leu
        35                  40                  45
Ala Lys Pro Asp Phe Gly Ala Glu Ala Lys Leu Glu Val Ser Ser Ser
    50                  55                  60
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PATENT COOPERATION TREATY

PCT

09/554933

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
660857		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/JP 98/ 05238	20/11/1998	25/11/1997
Applicant		
SAGAMI CHEMICAL RESEARCH CENTER et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readble form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP 98/05238

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see sheet B

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-6 in part (subject 1. on continuation-sheet)

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-6 in part

Protein comprising SEQ ID NO:1, DNA encoding it, cDNA comprising SEQ ID NO:4 or 7, expression vector and transformed eukaryotic cell capable of expressing the same.

2. Claims: 1-6 in part

Protein comprising SEQ ID NO:2, DNA encoding it, cDNA comprising SEQ ID NO:5 or 9, expression vector and transformed eukaryotic cell capable of expressing the same.

3. Claims: 1-6 in part

Protein comprising SEQ ID NO:3, DNA encoding it, cDNA comprising SEQ ID NO:6 or 11, expression vector and transformed eukaryotic cell capable of expressing the same.

INTERNATIONAL SEARCH REPORT

International Application No
CT/JP 98/05238

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 . C12N15/12 C07K14/47 C12N15/85 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JULIE E. REEVES ET AL.: "The surf-4 gene encodes a novel 30kDa intgral membrane protein" MOLECULAR MEMBRANE BIOLOGY, vol. 12, no. 2, April 1995 (1995-04), pages 201-208, XP002096695 page 207, left-hand column, paragraph 1 - right-hand column, last paragraph; figure 1 --- -/--	1-6

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 March 1999	14. 07. 99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	MONTERO LOPEZ B.

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLARE HUXLEY ET AL.: "The mouse surfeit locus contains a cluster of six genes associated with four CpG-rich islands in 32 kilobases of genomic DNA" MOLECULAR AND CELLULAR BIOLOGY, vol. 10, no. 2, February 1990 (1990-02), pages 605-614, XP002096696 WASHINGTON US figure 2 ---	1-6
A	YU FENG ET AL.: "HIV-1 entry cofactor: Functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor" SCIENCE, vol. 272, no. 5263, 10 May 1996 (1996-05-10), pages 872-877, XP002096721 cited in the application the whole document -----	1-6

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tcc tat gct gtc ttc gtt gtc acc tac ctg atc ctt gcc tgc tgc cag 545
Ser Tyr Ala Val Phe Val Val Thr Tyr Leu Ile Leu Ala Cys Cys Gln
140 145 150 155

gga ccc aga cgc cgt ttc cca tgg aac atc att ctg ctg acc ctt ttt 593
Gly Pro Arg Arg Arg Phe Pro Trp Asn Ile Ile Leu Leu Thr Leu Phe
160 165 170

act ttt gcc atg ggc ttc atg acg ggc acc att tcc agt atg tac caa 641
Thr Phe Ala Met Gly Phe Met Thr Gly Thr Ile Ser Ser Met Tyr Gln
175 180 185

acc aaa gcc gtc atc att gca atg atc atc act gcg gtg gta tcc att 689
Thr Lys Ala Val Ile Ile Ala Met Ile Ile Thr Ala Val Val Ser Ile
190 195 200

tca gtc acc atc ttc tgc ttt cag acc aag gtg gac ttc acc tcg tgc 737
Ser Val Thr Ile Phe Cys Phe Gln Thr Lys Val Asp Phe Thr Ser Cys
205 210 215

aca ggc ctc ttc tgt gtc ctg gga att gtg ctc ctg gtg act ggg att 785
Thr Gly Leu Phe Cys Val Leu Gly Ile Val Leu Leu Val Thr Gly Ile
220 225 230 235

gtc act agc att gtg ctc tac ttc caa tac gtt tac tgg ctc cac atg 833
Val Thr Ser Ile Val Leu Tyr Phe Gln Tyr Val Tyr Trp Leu His Met
240 245 250

ctc tat gct gct ctg ggg gcc att tgt ttc acc ctg ttc ctg gct tac 881
Leu Tyr Ala Ala Leu Gly Ala Ile Cys Phe Thr Leu Phe Leu Ala Tyr
255 260 265

gac aca cag ctg gtc ctg ggg aac cgg aag cac acc atc agc ccc gag 929
Asp Thr Gln Leu Val Leu Gly Asn Arg Lys His Thr Ile Ser Pro Glu
270 275 280

gac tac atc act ggc gcc ctg cag att tac aca gac atc atc tac atc 977
Asp Tyr Ile Thr Gly Ala Leu Gln Ile Tyr Thr Asp Ile Ile Tyr Ile
285 290 295

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Phe Thr Phe Val Leu Gln Leu Met Gly Asp Arg Asn
300 305 310

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<211> 311

<212> PRT

<213> Homo sapiens

<400> 10

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Asp Arg Asn Pro Leu Tyr Pro Gly Pro Leu Pro Pro Gly Gly Tyr Gly
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Gln Pro Ser Val Leu Pro Gly Gly Tyr Pro Ala Tyr Pro Gly Tyr Pro
30 35 40

Gln Pro Gly Tyr Gly His Pro Ala Gly Tyr Pro Gln Pro Met Pro Pro
45 50 55

Thr His Pro Met Pro Met Asn Tyr Gly Pro Gly His Gly Tyr Asp Gly
60 65 70 75

Glu Glu Arg Ala Val Ser Asp Ser Phe Gly Pro Gly Glu Trp Asp Asp
80 85 90

Arg Lys Val Arg His Thr Phe Ile Arg Lys Val Tyr Ser Ile Ile Ser
95 100 105

Val Gln Leu Leu Ile Thr Val Ala Ile Ile Ala Ile Phe Thr Phe Val
110 115 120

Glu Pro Val Ser Ala Phe Val Arg Arg Asn Val Ala Val Tyr Tyr Val
125 130 135

Ser Tyr Ala Val Phe Val Val Thr Tyr Leu Ile Leu Ala Cys Cys Gln

140 145 150 155

Gly Pro Arg Arg Arg Phe Pro Trp Asn Ile Ile Leu Leu Thr Leu Phe

160 165 170

Thr Phe Ala Met Gly Phe Met Thr Gly Thr Ile Ser Ser Met Tyr Gln

175 180 185

Thr Lys Ala Val Ile Ile Ala Met Ile Ile Thr Ala Val Val Ser Ile

190 195 200

Ser Val Thr Ile Phe Cys Phe Gln Thr Lys Val Asp Phe Thr Ser Cys

205 210 215

Thr Gly Leu Phe Cys Val Leu Gly Ile Val Leu Leu Val Thr Gly Ile

220 225 230 235

Val Thr Ser Ile Val Leu Tyr Phe Gln Tyr Val Tyr Trp Leu His Met

240 245 250

Leu Tyr Ala Ala Leu Gly Ala Ile Cys Phe Thr Leu Phe Leu Ala Tyr

255 260 265

Asp Thr Gln Leu Val Leu Gly Asn Arg Lys His Thr Ile Ser Pro Glu

270 275 280

Asp Tyr Ile Thr Gly Ala Leu Gln Ile Tyr Thr Asp Ile Ile Tyr Ile

285 290 295

Phe Thr Phe Val Leu Gln Leu Met Gly Asp Arg Asn

300 305 310

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<211> 3705

<212> DNA

<213> Homo sapiens

<400> 11

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ggc atg gca ggg att cca ggg ctc ctc ttc ctt ctc ttc ttt ctg ctc 168
Met Ala Gly Ile Pro Gly Leu Leu Phe Leu Leu Phe Phe Leu Leu
1 5 10 15

tgt gct gtt ggg caa gtg agc cct tac agt gcc ccc tgg aaa ccc act 216
Cys Ala Val Gly Gln Val Ser Pro Tyr Ser Ala Pro Trp Lys Pro Thr
20 25 30

tgg cct gca tac cgc ctc cct gtc gtc ttg ccc cag tct acc ctc aat 264
Trp Pro Ala Tyr Arg Leu Pro Val Val Leu Pro Gln Ser Thr Leu Asn
35 40 45

tta gcc aag cca gac ttt gga gcc gaa gcc aaa tta gaa gta tct tct 312
Leu Ala Lys Pro Asp Phe Gly Ala Glu Ala Lys Leu Glu Val Ser Ser
50 55 60

tca tgt gga ccc cag tgt cat aag gga act cca ctg ccc act tac gaa 360
Ser Cys Gly Pro Gln Cys His Lys Gly Thr Pro Leu Pro Thr Tyr Glu
65 70 75

gag gcc aag caa tat ctg tct tat gaa acg ctc tat gcc aat ggc agc 408
Glu Ala Lys Gln Tyr Leu Ser Tyr Glu Thr Leu Tyr Ala Asn Gly Ser
80 85 90 95

cgc aca gag acg cag gtg ggc atc tac atc ctc agc agt agt gga gat 456
Arg Thr Glu Thr Gln Val Gly Ile Tyr Ile Leu Ser Ser Ser Gly Asp
100 105 110

ggg gcc caa cac cga gac tca ggg tct tca gga aag tct cga agg aag 504
Gly Ala Gln His Arg Asp Ser Gly Ser Ser Gly Lys Ser Arg Arg Lys
115 120 125

cgg cag att tat ggc tat gac agc agg ttc agc att ttt ggg aag gac 552
Arg Gln Ile Tyr Gly Tyr Asp Ser Arg Phe Ser Ile Phe Gly Lys Asp
130 135 140

ttc ctg ctc aac tac cct ttc tca aca tca gtg aag tta tcc acg ggc 600
Phe Leu Leu Asn Tyr Pro Phe Ser Thr Ser Val Lys Leu Ser Thr Gly
145 150 155

tgc acc ggc acc ctg gtg gca gag aag cat gtc ctc aca gct gcc cac 648
Cys Thr Gly Thr Leu Val Ala Glu Lys His Val Leu Thr Ala Ala His
160 165 170 175

tgc ata cac gat gga aaa acc tat gtg aaa gga acc cag aag ctt cga 696
Cys Ile His Asp Gly Lys Thr Tyr Val Lys Gly Thr Gln Lys Leu Arg
180 185 190

gtg ggc ttc cta aag ccc aag ttt aaa gat ggt ggt cga ggg gcc aac 744
Val Gly Phe Leu Lys Pro Lys Phe Lys Asp Gly Gly Arg Gly Ala Asn
195 200 205

gac tcc act tca gcc atg ccc gag cag atg aaa ttt cag tgg atc cgg 792
Asp Ser Thr Ser Ala Met Pro Glu Gln Met Lys Phe Gln Trp Ile Arg
210 215 220

gtg aaa cgc acc cat gtg ccc aag ggt tgg atc aag ggc aat gcc aat 840
Val Lys Arg Thr His Val Pro Lys Gly Trp Ile Lys Gly Asn Ala Asn
225 230 235

gac atc ggc atg gat tat gat tat gcc ctc ctg gaa ctc aaa aag ccc 888
Asp Ile Gly Met Asp Tyr Asp Tyr Ala Leu Leu Glu Leu Lys Lys Pro
240 245 250 255

cac aag aga aaa ttt atg aag att ggg gtg agc cct cct gct aag cag 936
His Lys Arg Lys Phe Met Lys Ile Gly Val Ser Pro Pro Ala Lys Gln
260 265 270

ctg cca ggg ggc aga att cac ttc tct ggt tat gac aat gac cga cca 984
Leu Pro Gly Gly Arg Ile His Phe Ser Gly Tyr Asp Asn Asp Arg Pro
275 280 285

ggc aat ttg gtg tat cgc ttc tgt gac gtc aaa gac gag acc tat gac 1032
Gly Asn Leu Val Tyr Arg Phe Cys Asp Val Lys Asp Glu Thr Tyr Asp
290 295 300

ttg ctc tac cag caa tgc gat gcc cag cca ggg gcc agc ggg tct ggg 1080
Leu Leu Tyr Gln Gln Cys Asp Ala Gln Pro Gly Ala Ser Gly Ser Gly
305 310 315

gtc tat gtg agg atg tgg aag aga cag cag cag aag tgg gag cga aaa 1128
Val Tyr Val Arg Met Trp Lys Arg Gln Gln Gln Lys Trp Glu Arg Lys
320 325 330 335

att att ggc att ttt tca ggg cac cag tgg gtg gac atg aat ggt tcc 1176
Ile Ile Gly Ile Phe Ser Gly His Gln Trp Val Asp Met Asn Gly Ser
340 345 350

cca cag gat ttc aac gtg gct gtc aga atc act cct ctc aaa tat gcc 1224
Pro Gln Asp Phe Asn Val Ala Val Arg Ile Thr Pro Leu Lys Tyr Ala
355 360 365

cag att tgc tat tgg att aaa gga aac tac ctg gat tgt agg gag ggg 1272
Gln Ile Cys Tyr Trp Ile Lys Gly Asn Tyr Leu Asp Cys Arg Glu Gly
370 375 380

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<211> 383

<212> PRT

<213> Homo sapiens

<400> 12

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Cys Ala Val Gly Gln Val Ser Pro Tyr Ser Ala Pro Trp Lys Pro Thr
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Trp Pro Ala Tyr Arg Leu Pro Val Val Leu Pro Gln Ser Thr Leu Asn
35 40 45

Leu Ala Lys Pro Asp Phe Gly Ala Glu Ala Lys Leu Glu Val Ser Ser
50 55 60

Ser Cys Gly Pro Gln Cys His Lys Gly Thr Pro Leu Pro Thr Tyr Glu
65 70 75

Glu Ala Lys Gln Tyr Leu Ser Tyr Glu Thr Leu Tyr Ala Asn Gly Ser
80 85 90 95

Arg Thr Glu Thr Gln Val Gly Ile Tyr Ile Leu Ser Ser Ser Gly Asp
100 105 110
Gly Ala Gln His Arg Asp Ser Gly Ser Ser Gly Lys Ser Arg Arg Lys
115 120 125
Arg Gln Ile Tyr Gly Tyr Asp Ser Arg Phe Ser Ile Phe Gly Lys Asp
130 135 140
Phe Leu Leu Asn Tyr Pro Phe Ser Thr Ser Val Lys Leu Ser Thr Gly
145 150 155
Cys Thr Gly Thr Leu Val Ala Glu Lys His Val Leu Thr Ala Ala His
160 165 170 175
Cys Ile His Asp Gly Lys Thr Tyr Val Lys Gly Thr Gln Lys Leu Arg
180 185 190
Val Gly Phe Leu Lys Pro Lys Phe Lys Asp Gly Gly Arg Gly Ala Asn
195 200 205
Asp Ser Thr Ser Ala Met Pro Glu Gln Met Lys Phe Gln Trp Ile Arg
210 215 220
Val Lys Arg Thr His Val Pro Lys Gly Trp Ile Lys Gly Asn Ala Asn
225 230 235
Asp Ile Gly Met Asp Tyr Asp Tyr Ala Leu Leu Glu Leu Lys Lys Pro
240 245 250 255
His Lys Arg Lys Phe Met Lys Ile Gly Val Ser Pro Pro Ala Lys Gln
260 265 270
Leu Pro Gly Gly Arg Ile His Phe Ser Gly Tyr Asp Asn Asp Arg Pro
275 280 285
Gly Asn Leu Val Tyr Arg Phe Cys Asp Val Lys Asp Glu Thr Tyr Asp
290 295 300
Leu Leu Tyr Gln Gln Cys Asp Ala Gln Pro Gly Ala Ser Gly Ser Gly

305 310 315

Val Tyr Val Arg Met Trp Lys Arg Gln Gln Gln Lys Trp Glu Arg Lys

320 325 330 335

Ile Ile Gly Ile Phe Ser Gly His Gln Trp Val Asp Met Asn Gly Ser

340 345 350

Pro Gln Asp Phe Asn Val Ala Val Arg Ile Thr Pro Leu Lys Tyr Ala

355 360 365

Gln Ile Cys Tyr Trp Ile Lys Gly Asn Tyr Leu Asp Cys Arg Glu Gly

370 375 380